



A34263 – 072819.0128

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

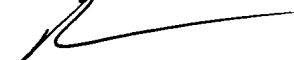
Applicants : Mc.Neil-Watson et al. Customer No.: 21003
Serial No. : 09/843,339 Examiner: To Be Assigned
Filed : April 26, 2001 Group Art Unit: 1743
For : MOBILITY AND EFFECTS ARISING FROM SURFACE CHARGE

CLAIM FOR PRIORITY UNDER 35 U.S.C. §119

I hereby certify that this paper is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

May 30, 2006
Date of Deposit

Paul A. Ragusa
Attorney Name


Signature

38,587
PTO Registration No.

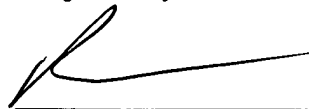
May 30, 2006
Date of Signature

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

A claim for priority is hereby made under the provisions of 35 U.S.C. §119 for the above-identified U.S. patent application based upon Great Britain Application No. GB 0010377.0 filed April 29, 2000. A certified copy of this application is enclosed. The Great Britain application is identified in the Declaration of the application Serial No. 09/843,339.

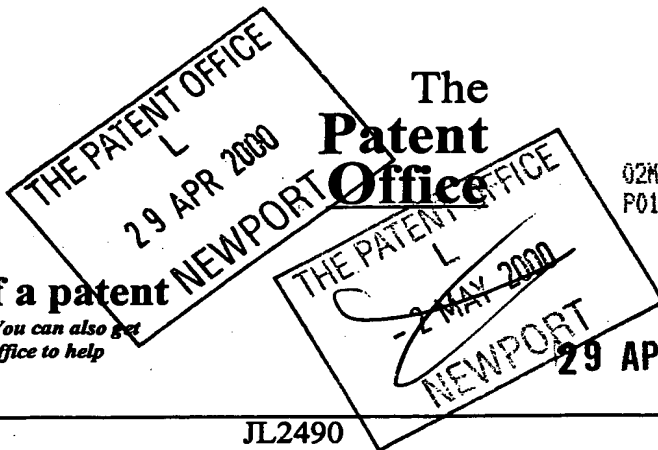
Respectfully submitted,



Date: 5-30-2006

Paul A. Ragusa
Patent Office Reg. No. 38,587
Attorney for Applicants
(212) 408-2588

THIS PAGE BLANK (USPTO)



The Patent Office

02MAY00 E533116-1 D02806
P01/7700-0.00-0010377.0

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet, from the Patent Office to help you fill in this form)

The Patent Office

Cardiff Road
Newport
Gwent NP9 1RH

1. Your reference JL2490

2. Patent application number
(The Patent Office will fill in this part) 0010377.0

3. Full name, address and postcode of the or of each applicant (underline all surnames)
MALVERN INSTRUMENTS LIMITED
ENIGMA BUSINESS PARK
GROVEWOOD ROAD
MALVERN
WORCESTERSHIRE WR14 1XZ

Patents ADP number (if you know it) 583963002
If the applicant is a corporate body, give the country/state of its incorporation ENGLAND

4. Title of the invention MOBILITY AND EFFECTS ARISING FROM SURFACE CHARGE

5. Name of your agent (if you have one) Barker Brettell
"Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode) 138 Hagley Road
Edgbaston
Birmingham
B16 9PW

Patents ADP number (if you know it) 7442494002 ✓

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and (if you know it) the or each application number

| Country | Priority application number (if you know it) | Date of Filing (day/month/year) |
|---------|--|---------------------------------|
| | | |

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

| Number of earlier application | Date of filing (day/month/year) |
|-------------------------------|---------------------------------|
| | |

8. Is a statement of inventorship and of right to grant of a patent required in support of this request (Answer 'Yes' if:
a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d)) YES

Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document
- Continuation sheets of this form -

Description 27 + 27

Claim(s) 07 + 07

Abstract -

Drawing(s) 09 + 09

10. If you are also filing any of the following, state how many against each item.

Priority documents

Translations of priority documents

Statement of inventorship and right to grant of a patent (*Patents Form 7/77*)

Request for preliminary examination
(*Patents Form 9/77*)

Request for substantive examination
(*Patents Form 10/77*)

Any other documents
(*please specify*)

11.

I/We request the grant of a patent on the basis of this application.

Signature

Barker Brettell

Date

28.04.00

12.

Name and daytime telephone number of person to contact in the United Kingdom

JOHN LAWRENCE

Tel: 0121 456 1364

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

- If you need help to fill in this form or you have any questions, please contact the Patent Office on 01645 500505
- Write your answers in capital letters using black ink or you may type them.
- If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.
- If you have answered 'Yes' *Patents Form 7/77* will need to be filed.
- Once you have filled in the form you must remember to sign and date it.
- For details of the fee and ways to pay please contact the Patent Office.

Patents Form 1/77

MOBILITY AND EFFECTS ARISING FROM SURFACE CHARGE

This invention relates to characterising or measuring mobility and effects arising from surface charge especially but not exclusively charges on particles, or charges associated with a test cell wall. It is especially
 5 useful in the field of electrophoretic mobility, measurements for particles using a capillary cell.

The general technique of particle electrophoresis is basically concerned
 10 with providing a suspension of fine particles or biological cells and subjecting it to a precise electric field, and hence causing the particles to attain a steady state velocity due to the charge at the surface of the particle. The surface charge may arise from ionisation of surface chemical groups or absorption of external matter, molecules or ions.
 15 Measuring this velocity can in principle allow determination of the zeta potential, which arises from the surface charge and the associated layer of ions that cluster near the surface. The velocity is often expressed as a velocity in unit electric field, which is then referred to as electrophoretic mobility.

20 If the surface or nature of the specimen is heterogeneous, different particles may have different charges per unit area and then the velocity will be different, and a spectrum of velocities may enable a more detailed characterisation of the specimen.

25 It is sometimes desired to know the electrophoretic mobility of a particle, or more likely the distribution of electrophoretic mobilities of particles. There are several prior art ways of measuring electrophoretic mobility of particles. These include direct optional observation where a user applies
 30 a DC electric field to a liquid sample that contains particles and observes

them using a microscope to see how long they take to travel a given distance. From a knowledge of the velocity of the particles in the electric field and a knowledge of the strength of the electric field the electrophoretic mobility can be determined. From the mobility it is also possible to derive other useful properties of the particles, such as their zeta potential.

It is known to perform direct optical measurements of particle velocity in a bath-like sample where the electrodes are immersed directly into the liquid sample, and to perform the measurements using a capillary cell where the liquid sample dispersion is provided in a capillary (optically transparent so that the user can see the particles), and the electric field is applied axially of the capillary tube. This enables the electrodes to be provided at either end of the tube and hence kept remote from the measuring region.

The capillary also allows precise regulation of the electric field and good temperature regulation of the specimen.

The particle distribution velocity measurement is made at a certain region in the capillary cell. This is defined by the optical system. In a simple image analysis system (which is essentially a microscope) the observation consists in timing the movement of particles in a suspension in order to record the velocities of enough particles to characterise the distribution of velocities, and hence electrophoretic mobilities.

The applied field is usually periodically reversed in polarity to avoid electrode degradation and permanent loss of particles from the measuring region. The inverse of this period will be referred to as the frequency of the field.

The fluid that contains the particles will usually have ions and these ions will move under the influence of the applied electric field. The movement of the ions can effectively provide a current of liquid and the observed speed of movement of the particles dispersed in the liquid is dependent upon their charge/size and the local flow of liquid at the point where the velocity measurement is made.

In a capillary cell it is noted that at low frequencies of applied field (around 1 Hz) the velocity of the particles is dependent on the position of the point of observation. It follows an apparently parabolic form in the region away from the cell walls. This is due to liquid flows set up by the effect of electric charge on the internal walls of the capillary. This flow is known as electro-osmotic flow, and its consequences are the major difficulty to overcome in exploiting the undoubted advantages of the capillary format. This is well known in the prior art. Methods used have included coating the cell walls with a neutral material and measuring at the so-called stationary layer - where the osmotic flow and counter flow cancel.

20

However, measuring at the stationary layer can be far from straightforward. Theoretically the stationary layer is an infinitely thin sheet, and so the best that can be achieved is to use a measurement zone which encompasses the stationary layer, and is relatively small (e.g. 50 μ m inside by 50 μ m tall by 50 μ m deep). A major problem is aligning the optical measurement system relative to the capillary tube accurately enough, and reliably, to ensure that it is taking measurements from the correct small region at the stationary level. A small error in the alignment of the optics of the measurement device can make the results obtained unreliable. At present it can take a long time to ensure that the

30

optics of the measurement apparatus are correctly aligned, and it can require a skilled and experienced operator to achieve proper alignment (e.g. to within 5 μm).

- 5 It is also known to use a laser beam to illuminate only a small region of the capillary, both to provide illumination for the particles and enhanced positioned selection. It is then necessary to align both the laser beam and the viewing/detector system with the same point in space and ensure that that point is at the stationary level, which is even more awkward. This
10 requires close engineering tolerances and dust free manufacturing techniques.

The above problem has led to the development of a high frequency (e.g. ≥ 100 Hz) field switching technique. If the direction of the electric field
15 is switched at high frequency it has been found that this reduces the electro-osmotic flow to a negligible amount, and so careful alignment to measure at the stationary level is no longer necessary. This technique is sometimes referred to as the Fast Field Reversal (FFR) electrophoretic mobility measurement technique.

20

This rapid switching can only be performed in an instrument which uses automatic methods of data collection, and the limited time for which the field is constant has a direct effect on the ability to distinguish or resolve differences in mobility.

25

Since in high frequency measurements particles are only observed for a short time travelling in only one direction differences in velocity distribution between the particles need to be bigger in order to be resolved: that is to say velocity differences below a threshold difference
30 will not be resolvable or will be very difficult to resolve. Therefore the

particle electrophoretic mobility distribution will be less accurately resolved (but alignment is not such a problem).

According to a first aspect the invention comprises a method of
5 automatically determining an electric charge - related characteristic or
derived parameter of particles in a dispersion or of a cell wall, comprising
having a particle - containing dispersion provided in a cell; illuminating
the dispersion with light from a light source; detecting from a detection
volume scattered from the particles; applying an electric field to the
10 dispersion at a first frequency of change of direction of the electric field;
applying an electric field to the dispersion at a second frequency of
change of direction of the electric field; using first signals detected from
the scattered light when the first frequency electric field is applied to
provide first values related to the velocity distribution of the particles;
15 and using a second velocity related signal or value derived from scattered
light during the time that the second frequency electric field is applied to
modify the velocity distribution related values to produce a modified
particle velocity-related distribution; and wherein the first frequency is
low enough to obtain an acceptable resolution of the distribution of
20 particle velocity - related values.

The second frequency is high enough that the second velocity-related
signal or signals is substantially invariant on the precise position in the
cell at which the detection volume is located.

25

The modification of the first values using the second signal value
substantially compensates the first signal values for a shift in value that
would otherwise be present dependant upon the position of the detection
volume.

30

- According to another aspect the invention comprises a method of determining the electrophoretic mobility of particles in a dispersion comprising having the dispersion in a capillary cell and taking particle velocity related measurements at a relatively high frequency of reversal of an applied electric field taking particle velocity measurements from the dispersion in the cell at a DC or relatively low frequency of field reversal to enable a relatively high resolution of particle velocity distribution to be obtained in comparison with the resolution that could be obtained using only measurements at the high frequency of field reversal; and using the high frequency measurements to shift the particle - velocity related distribution spectra obtained at low frequency so that the mean of its low frequency peak or peaks in the low frequency spectra substantially coincide with the position of the means of the corresponding high frequency spectral peaks, thereby producing a translated, modified, spectrum substantially equivalent to having peaks of the low frequency shape present at the positions of the equivalent high frequency peaks; and using the modified spectra to determine or indicate the electrophoretic mobility of the particles in the dispersion.
- The high frequency of reversal of the electric field is such that the variation in mean velocity for a particle peak in the particle velocity distribution is substantially invariant with the precise position in the cell from which measurements are taken.
- According to another aspect the invention comprises a method of determining electrophoretic values for particles in a dispersion held in a capillary, the method comprising taking particle velocity-related measurements at a high enough frequency of electric field reversal such that the effect of electro-osmotic flow of the dispersion is negligible; taking velocity - related measurements at a low enough frequency of field

reversal such that electro-osmotic flow has a significant effect on the measured velocities, depending upon where in the cross-section of the capillary is the observed signal - generating region, and providing an offset to the low frequency measurements to offset the effect of the electro-osmotic flow part of the observed low frequency velocities, the offset being derived by using the high frequency measurements.

According to another aspect the invention comprises the measurement of electrophoretic mobility of a dispersion of particles or cells, the measurement being made at or near to the centre of the cross-section of a capillary cell using an optical technique that can select a sample volume that is a small fraction of the cross-section, and in which the frequency of the applied electric field is varied between high and low levels, high level being the frequency at which the electro-osmotic flow of fluid due to the electric charge on the cell wall is substantially zero at the centre of the cell and the low level being such that the parabolic flow characteristic of electro-osmosis is substantially fully established.

The particles could be biological cells.

The form of the applied field can be sinusoidal or of a square wave, or any other known shape depending on the nature of the signal processing applied.

In any preceding invention the measurement of the mean mobility of a distribution of one or more mobilities made at the high frequency may be used to adjust the spectrum of mobilities measured at the low frequency so that the mean of distribution is made identical or similar. The adjustment required may be a linear offset of the spectrum. The size and magnitude of the offset may constitute a determination of the electro-osmotic flow

velocity. The adjustment could be made directly to the optics (for example by use of an optical modulator) prior to signal capture, rather than after signal capture.

- 5 It is possible to use a conversion of electrophoretic mobility and electro-osmotic velocity to provide a simultaneous determination of the zeta potential distribution of the particle dispersion and/or the zeta potential of the wall of the capillary.
- 10 Measurements may be taken at more than two electric field frequencies, possibly of the order of several, or many, (e.g ten or tens of) frequencies.

According to another aspect the invention comprises a capillary particle electrophoretic mobility distribution determining apparatus comprising:

- 15 a holder for a capillary cell adapted to contain a dispersion;

a light source;
- 20 a detector adapted to detect light scattered from a detection zone of the capillary;

electric field generating electrodes adapted to generate an electric field;
- 25 a controller adapted to control the electric field applied by the electrodes;

a signal processor adapted to process the signals detected in use by the detector to determine a velocity mobility distribution; and in which

the controller is adapted to apply an electric field at a first, relatively low, frequency and at least a second relatively high frequency, the first frequency being low enough that better velocity distribution resolution is achieved in use than could be at the second frequency, and the second
5 frequency being high enough that the measured velocity distribution is substantially unaffected by electro-osmotic flow; and in which the processor is adapted in use to modify the particle velocity distribution spectra obtained at the first frequency by shifting it by an offset amount to remove the electro-osmotic velocity, the offset amount being determined
10 using information from measurements at both the first and second frequencies of field renewal.

Preferably the detection zone is adapted to be substantially at the centre of the cross-section of the capillary. The light source is preferably a laser
15 and laser doppler velocimetry is preferably used to obtain the first and/or second particle velocity distribution.

It will be appreciated that the velocity distribution is one-to-one related to the electrophoretic mobility distribution (divide by the strength of E at the
20 measurement zone (which is known for any particular electrode voltage setting from any particular machine) to get velocity in unit field strength) and the scope of protection is intended to cover both substantively interchangeably.

25 Preferably a capillary cell is present in the apparatus, but the apparatus may be sold/provided with no cell attached, the cell being obtained separately and fitted to the apparatus when full of sample.

Preferably the high frequency measurements are used to establish a mean
30 velocity for a measured spectrum and the mean velocity of the spectrum at

low velocity is used to determine the offset value (measured mean velocity of the spectrum at low frequency minus mean velocity of the spectrum at high frequency equals the electro-osmotic offset).

- 5 The first frequency may be $1\text{Hz} \pm 1\text{Hz}$, or $\pm 0.5\text{Hz}$. It may be substantially 1Hz . The second frequency may be $100\text{ Hz} \pm 30\text{Hz}$, or $\pm 15\text{Hz}$, or ± 10 or $\pm 5\text{Hz}$. Alternatively the second frequency could be significantly more than 100Hz , for example $300, 400, 500, 600\text{ Hz}$ or more.

10

The apparatus preferably has scattered light collecting optics and an optical alignment adjustment mechanism (or the user can preferably manually control it) to locate the detection zone to $200\mu\text{m}$ or better, preferably to about $50\mu\text{m}$.

15

Similarly the light source preferably has a light source alignment mechanism that is manually or controller processor computer controlled to align a beam of light at the detection zone to $200\mu\text{m}$ or better, preferably to about $50\mu\text{m}$. The volume of the detection zone is preferably $100\mu\text{m} \times$

20 $100\mu\text{m} \times 100\mu\text{m}$ (eg. $10^6 \mu\text{m}^3$).

The controller may apply a square wave field or a sinusoidal field. The detected signals are preferably gated to ignore detected signals generated at a time close to the change of direction of movement of the particles.

25

The high frequency is preferably high enough to create a substantially flat parabola for the electro-osmotic flow, or at least substantially flat at the central region of the cross-section (from where the signals are detected).

The apparatus preferably has a display, printer, or output port adapted to disseminate information relating to the particle velocity distribution and/or electrophoretic mobility distribution and/or zeta potential distribution.

5

According to another aspect the invention comprises a data carrier embodying a computer programme which when run on a capillary electrophoretic mobility analyser performs the method of any aspect of the invention and/or creates apparatus in accordance with another aspect of

10

the invention.

According to another aspect the invention comprises a method of determining charge-related characteristics of either (i) particles in a dispersion, or (ii) the material of a test cell wall, or (iii) particle-cell wall

15

attraction; the method comprising providing a fluid sample having particles in it in a cell having cell walls and applying an electric field which is DC or which alternates direction at a relatively low frequency and taking DC/low frequency particle velocity measurements, and also taking particle velocity measurements when the electric field is applied at

20

a substantially higher frequency; and using both the high frequency and low frequency measurements to determine the charge - related characteristic.

25

Preferably the measurements are taken from the centre of the cross-section of the cell and preferably the cell is a capillary.

30

We have appreciated that it is not necessary either to get good resolution at low frequency/DC but take great care to find the stationary level or to get easy to obtain/set up high frequency spectra with poorer velocity resolution, but that it is possible to use high frequency information to

modify low frequency spectra to get a combined/modified high frequency spectra with alignment – insensitive set up. We have realised that at high frequency the velocity of the spectra taken at the centre of a capillary and at the stationary level are not shifted significantly as electro osmosis is negligible, and that it is possible to replace the broadened spectra with the sharper spectra from low frequency measurements.

An embodiment of the invention will now be described by way of example only with reference to the accompanying drawings of which:-

10

Figure 1 illustrates schematically a prior art direct optical measurement of the velocity of particles in a capillary moving under the influence of an electric field;

15

Figure 2 illustrates schematically the phenomenon of electro-osmotic flow in a capillary and indicates the stationary level;

Figure 3 illustrates schematically an instrument in accordance with the invention;

20

Figure 4 illustrates the effect of measuring particle velocities at two different places in the cross-section of the capillary using a low frequency electric field;

25

Figure 5 illustrates the effect of measuring particle velocities at two different places in the cross-section of the capillary using a high frequency electric field;

Figures 6 to 8 show schematically the effect of the invention;

30

Figure 9 shows a schematic diagram of an opto-electrical frequency offset apparatus according to the present invention;

Figures 10A and 10B show a schematic process flow for the apparatus of Figure 3;

Figure 11 shows equations used by the apparatus of Figure 3 to produce the particle electrophoretic mobility distribution; and

Figure 12A and 12B show typical high frequency and low frequency particle mobility spectrum respectively; and

Figure 13 shows a plot of mobility versus frequency for a particle in a specified medium.

Figure 1 shows a microscope 10 collecting light from a region 12 of a capillary test container 14. The capillary 14 has transparent walls 15 and contains a liquid 16 having a dispersion of particles 18 suspended in it. Observations are taken (e.g. normally by the eye, or with a digital camera and appropriate particle-tracking software, or with a photographic film camera and post-development processing) to determine the velocity of a number of particles, and hence determine a particle velocity distribution and/or an electrophoretic mobility distribution for the particles. The microscope 10 could, of course, be some other viewing system or optical detector.

Figure 2 shows the phenomenon of electro-osmotic flow. This is the movement of the liquid supporting the particles dispersion due to the presence of ions, which are also transported by the electric field. The distribution of ions is affected by the charge that exists on the walls of the

capillary tube and hence the net flow of liquid (and particles suspended in the liquid) depends upon distance from the wall of the capillary. The liquid flows at the periphery of the capillary in one direction, referenced 22, and at the central region of the capillary in the opposite, return, direction, referenced 20. (It has to return or else there would be a build up of liquid at one end of the cell, and the cell is full of liquid and has no space). The capillary cell wall 15 is negatively charged (reference 24) and this causes positive ions 26 to be in excess in the liquid at the inner surface of the cell wall. Line 30 of Figure 4 illustrates the velocity of the liquid in the capillary cell. There is a tubular sheet surface, referenced 32, where the peripheral flow and the central counter flow meet and the velocity of the liquid is zero: this is the stationary level where in the prior art technique of low frequency capillary cell electrophoretic mobility measurements it is necessary to measure the particle velocity distribution (or attempt to do so). The position of the stationary layer can be calculated for simple shapes of capillary (see R.J. Hunter, Zeta Potential in Colloid Science, Academic Press 1981, p 131-134).

Figure 4 shows a graph of normalised intensity of detected scattered light (I) against measured particle velocity for two points of measurements in the cross-sectional area of the capillary cell. The intensity of the scattered light is usually measured as photon counts per second, however it may be measured as a voltage or current which is proportional to the intensity of the scattered light. Alternatively, if image analysis is performed the intensity measurement may take the form of a particle count. Line 40 is related to measurements from the stationary layer, referenced 41, and line 42 is related to measurements from the centre of the capillary tube, referenced 43. As will be seen the detected peak when measured at the centre of the capillary is found at a different measured

velocity (V_2) to that when measured at the stationary level (V_1). This velocity shift is related to the electro-osmotic flow.

Figure 4 shows the measurements when a low frequency electric field is applied, for example 1Hz.

Figure 5 shows equivalent information to Figure 4, but for a high frequency electric field, for example 100 Hz. Similar features have been given similar reference numbers, with the stationary layer velocity being referenced as V_3 and the cell centre velocity as V_4 .

The velocities V_1 and V_3 are the velocities at the stationary layer and are relevant for establishing electrophoretic mobility. Velocities V_2 and V_4 are the velocities of particles at the centre of the cell, and are substantially the sum of the electrophoretic mobility velocity and the electro-osmotic velocity. $V_2 - V_1$ equals the liquid flow at the centre of the cell (or at any other point where V_2 is measured). Similarly, $V_4 - V_3$ equals the electro-osmotic flow at the point of measurement.

It will be noted that W_1 in Figure 4 and W_2 in Figure 5 represent the width of the velocity spectrum measured. In Figure 4, with low frequency E , the velocity spectrum is narrow and precise information is obtained. In Figure 5, with high frequency E , W_2 is broader, giving poorer resolution of the velocity of a particle/poorer resolution of a distribution of velocities of a distribution of particles.

It will be noted that in Figure 4 the rate of change of the fluid velocity at the region of the stationary level is large (indeed the rate of change of fluid velocity with spatial position is greatest there). This means that any small error in position at which the measurement is made effects the

measured value for V_1 significantly. On the other hand, the rate of change of fluid velocity at the stationary level in Figure 5, with high frequency, is relatively low, and the measured value of V_3 is relatively position-insensitive. Indeed, there is not much difference between V_3 and V_4 (and this difference is the maximum variation in measured particle velocity due to fluid flow variations). Figure 4 has a steep parabolic curve, especially steep at just the area where it is desired to measure in the prior art low frequency technique, and Figure 5 has an almost flat curve. This is why developments in high frequency electrophoretic mobility measurements are used. It does not matter much about alignment of the system in high frequency measurements.

Figure 3 shows an electrophoretic mobility measuring device or instrument 50 having a transparent capillary cell 52, a pair of electrodes 53 and 54 generating an electric field E ; a power supply or voltage control system 56 controlling the voltage of the electrodes; a laser 58 and laser alignment mechanism 60; a scattered light detection system 61 comprising collection/focusing optics 62, detector 64, and detector/optics alignment mechanism 66; and control/processor 68.

The electrodes 53 and 54 are held in chambers 70 and 71 defined by chamber housings 72 and 73. Seals 74 connect the chamber housings to the cell 52 to form a closed volume for dispersion 76 which has a liquid 77 and particles 78 dispersed in the liquid.

The cell 52 is typically made of silica/quartz and is about 5cm long by about 1cm wide by about 1½ cm tall, and has a rectangular cross-section capillary bore 80 extending axially along its centre, the capillary hole having a cross-sectional size of about 5mm x 2mm.

The laser alignment mechanism 60, and of the optics/detector alignment mechanism 66 are preset during manufacture to ensure that a laser beam 82 produced in use passes through (intersects) what is roughly the centre of the cross-section of the capillary bore 80, and to ensure that the optics 62 of the detection system 61 observes substantially and scattered light coming from a small volume of liquid at the centre of the capillary bore 80, where the laser beam 82 intersects the capillary (enhanced position selection). It will be appreciated that the laser beam 81 and the central optical axis of the detection system 61 may be orthogonal to each other, and orthogonal to the central axis of the capillary 52. The electric field extends along the central axis of the capillary 52.

The controller 68 controls the power supply (or voltage control system) 56 such that it produces an electric field that switches direction periodically. The controller is arranged to switch the electric field at two (or more) different frequencies. In the example of Figure 3 the controller can select between a high field frequency (eg 100Hz) or a low field frequency field (e.g. 1Hz). In the preferred embodiment shown a square wave electric field of frequency between 0.5Hz and 100 Hz is applicable to the sample.

The controller 68 also receives detected scatter signals (received from a viewing volume) from the detector 64 and analyses them to produce a particle electrophoretic mobility distribution for the sample 76 of liquid that has been introduced into the capillary 52. The detector does not image the particles, instead it uses whole field signals and monitors variations in detected intensity and looks for the doppler shifted field (doppler shift is related to velocity).

The control processor 68 effectively takes the signals representative of V_2 (cell centre measurement at low frequency, indicative of combined electrophoretic velocity of the particle plus electro-osmotic velocity of the liquid) and the signals representative of V_4 (cell centre measurement at high frequency, indicative of combined electrophoretic velocity of the particles plus electro-osmotic velocity of the liquid) and uses both signals to produce a "mixed mode" value indicative of the electrophoretic mobility dispersion of the particle, with substantially no element resulting from electro-osmotic flow.

10

This mixed mode, or compensated, electrophoretic mobility value is in this example achieved by subtracting the value of the centre of the V_4 peak, V_4 from the signals of the velocity distribution of particles measured at low frequency (V_2). This can be considered to be re-basing the zero origin of the velocity values for the low frequency measurements - introducing an offset on the velocities. The offset is what offset it takes to take the centre of the V_2 peak, to the value of V_4 , which as Figure 5 illustrates is substantially the same as V_3 (the stationary layer). This effect is $V_2 - V_4$. [$V_2 - (V_2 - V_4) = V_4$] It will be appreciated that V_2 will really be a distribution of velocities, each slightly different, and that V_4 will be a particular number.

Thus the instrument can be considered to take the high resolution V_2 peak (or more accurately distribution of peaks resulting from different particles) , which have an associated offset from the real electrophoretic velocities due to not being measured at the stationary layer, and subtracting the electro-osmotic flow contribution from their velocity by subtracting ($V_2 - V_4$). But V_2 is actually a range of velocities at high resolution, and V_4 is to be just the single velocity that is the centre of the V_4 peak, so this results in a translation of the V_2 distribution, at high

30

resolution, to the V_4 position (which is practically the same as the V_3 , stationary layer, position).

In this way we can have good resolution of the particle velocity
5 distribution (and have good resolution of the electrophoretic mobility
distribution) by measuring in a position -insensitive way within the cross-
section of the capillary. We can have the benefits of both high frequency
capillary electrophoretic mobility measurements (positional errors not
very significant) and the benefits of low frequency capillary
10 electrophoretic measurements (better resolution).

It will be appreciated that although we choose to measure at the central
axis of the capillary, the technique works for measurements taken
anywhere in the cross-section of the capillary (although we may not wish
15 to measure too close to the cell wall).

It will be appreciated that in our preferred embodiment we use the
technique of laser doppler velocimetry in which the coherent light source
produces scattered light which is mixed with a separated portion of the
20 coherent light source to measure the velocity using the doppler technique.
It is also possible to achieve a similar result using the doppler difference
method in which scattered light from two beams is detected. If an
incoherent light source was used we could image particles and process the
images to establish its velocity.

25

Figure 6 illustrates the principle of the invention. The differences Δ in
velocity of the centre line 90 of the low frequency peak and the centre
line 91 of the high frequency peak is closely associated with the effect
that the electro-osmotic flow has on the measured velocity of the
30 particles. This is subtracted from the low frequency velocity values to

give a distribution of adjusted velocities, which is substantially the same as would be measured had the measurements been taken at the stationary level with no fluid flow.

- 5 Figure 7 illustrates a particle velocity distribution, with different particles moving at different velocities. We may evaluate Δ for one peak and subtract the same value of Δ from each low frequency velocity value, or alternatively we may recalculate Δ for each pair of matched peaks.
- 10 Figure 8 shows the position where the peaks 92, 93 and 94 and 95 are so close together that at high frequency, low resolution, they cannot easily be resolved.

It will be appreciated that the relative heights of the graph lines for
15 high v , low v , and modified distribution graph in Figures 6 to 8 are not to be taken as an indication of actual relative intensities - we have spread them out in I value on the graph so that they can be more easily visualised.

- 20 Figure 9 illustrates the use of the direct comparison of a measured signal which has a frequency offset imposed upon it to a reference signal prior to electronic acquisition of data from the apparatus.

The frequency adjustment apparatus 100 comprises a laser light source
25 101, a beam splitter 102, a reference cell 103, an optical modulator 104, a measurement cell 105 and a comparator unit 106.

The laser light source 101 emits a beam of light 107 which is split into first and second light beam portions 107a, 107b by the beam splitter 102.

The first beam portion 107a passes through the reference cell 103 where both low and high frequency particles velocity measurements are made. The difference between the low and high frequency particle velocity measurements is stored, as an offset frequency value, and is indicative of the electro-osmotic flow characteristics of the system.

The offset frequency value is passed to the optical modulator 104.

The second beam portion 107b passes through the optical modulator 104 where it has an offset frequency equivalent to the offset frequency received from the reference cell 103 imposed upon it.

The second beam portion 107b then passes through the measurement cell 105 where high resolution, low frequency particle velocity measurements are made.

Both of the beam portions 107a, 107b are passed to the comparator unit 106 wherein a comparison between the signals can be made prior to signal capture.

It will be appreciated that in many embodiments we find only the nominal centre of the cross-section of the capillary and use the high frequency measurements to find the average peak position and the low frequency signals to provide the detail of the shape and/or height of the peak. The centre of the defined peak is used as the centre of the transposed low frequency peak (in one embodiment).

We may measure, using our apparatus, particle distributions when the particles are from $0.05\mu\text{m}$ to $50\mu\text{m}$ in dimension.

We believe that finding the centre of the capillary can now be done to $200 \pm 100\mu\text{m}$, which is far less onerous than the prior art low frequency technique where an accuracy of $\pm 5\mu\text{m}$ was required. The strength of our electric field is set by the electrodes and the length of the capillary.

- 5 The cross-section of the capillary sets the ion current flow, and hence can be used to control the heating of the sample (by ensuring a low ion flow).

Since the basis of the method is to subtract out the electro-osmotic velocity the high frequency that is used is high enough that the electro-
10 osmotic velocity is low enough to be ignored. This will usually be at a frequency of 80Hz or more, or 100Hz, or 200Hz +.

If we use too high a frequency and/or use too strong an electric field dielectrophoresis may occur, which will give erroneous results.

15

When the electric field is reversed the particles change direction. At the time of change of direction of movement, and around that time, the velocity of the particles is not constant (they are accelerating/decelerating). The detected signals are therefore normally
20 gated around the transition points to ensure that the signals that are counted (those that affect the output of the calculations) are from the terminal velocity periods of movement of the particles only.

A practical target is to achieve an average of about 2%, or better,
25 variation from the "true" velocity for a particle.

The system may use more than two frequencies for E field reversal, possibly about 3, 4, 5, 10 or more, or 20 or more.

Figure 13 shows a typical mobility versus frequency plot for a particle whose velocity measured in an apparatus, and/or using a method, according to the present invention.

- 5 At low frequencies, typically less than 2Hz, the electro-osmotic flow pattern is substantially parabolic between the walls of a capillary cell, and at higher frequencies, of the order of a few tens of Hz, it is effectively constant across the cell.
- 10 However, in the case of intermediate frequencies the electro-osmotic flow pattern will be a variable combination of the low and high frequency flow patterns.

Therefore, the use of a low and high frequency particle velocity
15 measurements (points A and B respectively on Figure 13) significantly reduces the computational complexity involved in calculating the frequency offset required to compensate for the electro-osmotic flow.

However, if the functional form of the electro-osmotic flow patterns at
20 intermediate frequencies is known, it may be possible to use two or more particle velocity measurements, at least one of which (or two or more) may be made at intermediate frequency values (see for example C1, C2, C3 on Figure 13) to calculate the frequency offset.

25 The calculation of the frequency offset at intermediate frequency values will require the deconvolution of the electro-osmotic flow pattern from the particle velocity measurements and the extrapolation of the mobility versus frequency curve in order to obtain the low and high frequency measurements and hence the difference therebetween.

We may interleave high frequency measurements with low frequency measurements. This may help to avoid distortion of the evaluated results due to the character of the particles changing (compared to if we did several high v measurements and then several low v measurements). We
5 may measure high v , low v pairs of measurement a number of times, say, 10 times and take an average, or integrate the signals from the high v measurements and those from the low v measurements and operate on the integrated values to determine the velocity/mobility distribution.

10 It is possible to measure at any point in the cross-section of the capillary for electrophoresis mobility measurements, but the measurements may also enable us to measure one or more characteristics of the cell wall. It would be better to measure generally in the middle of the capillary cross-section to obtain data indicative of all cell wall information.

15 The invention combines a measurement at high frequency that measures the mean mobility of the dispersion, with a measurement at low frequency that establishes the details of the spectrum. The amount by which the latter is adjusted to predict the same mean value is a measurement of the
20 effective state of charge of the cell wall. This is secondary, but potentially useful information.

It will be appreciated that the calculations and algorithms needed with the present invention are substantially the same as those needed with
25 conventional laser doppler shift capillary electrophoretic mobility algorithms/calculations.

In one embodiment it is possible to simply calculate the DC or low frequency distribution shape and locate the centre of the low frequency
30 spectrum on the centre of the high frequency spectrum (calculating both

from observed scattered light data) to provide a composite result containing high resolution electrophoretic mobility spectra.

It is possible to use information about the charge characteristics of the cell wall to: (a) determine whether the cell wall is dirty and needs cleaning (and have the apparatus provide an automatic indication of this to the user); (b) determine the extent to which the particles in the dispersion stick/adhere to the cell wall, which can tell us additional information about the cell wall, or the particles, or both; make the cell wall out of a material in which we are interested (e.g. coat it with that material) and determine charge characteristics of the material.

Of course, it is also possible to use the velocity measurements and/or the electrophoretic mobility measurements to determine the zeta potential of the particles, and the machine may do that automatically. It may be capable of producing a display or print out of zeta potential distribution.

Although it is apparent from the foregoing, the calculation process used by the particle electrophoretic mobility distribution analysis machine will be described, with reference to Figures 10 to 12.

The machine sets the amplitude of the applied electric field, either from a pre-stored programme, or the user can input it/select it. The machine then sets the frequency of the high frequency to be used (again either automatically or user selected from available options, or user input). The machine collects intensity signals at the high frequency E field and computes the auto correlation function of the signal. The machine collects light until a required amount of light, or time, has been achieved. The process of collecting light constitutes recording successive samples integrated for, typically, 100 to 1000 microseconds, and storing these

samples in a memory buffer. The conversion from light to electrical signals representative of intensity may be analogue, and may use ADC converter to get digital electronic signals, or digital (in which case individual photons are counted).

5

An autocorrelation function is created from the stored samples and once the data collection/creation of the autocorrelation function for high frequency is complete the frequency distribution is converted to a velocity mobility distribution by performing a Fourier Transform on the correlation function, and the velocity/mobility distribution is stored as a high frequency velocity/mobility distribution.

It will be appreciated that the autocorrelation function process is used to integrate the signal before later performing a Fourier Transform, but it is alternatively possible to perform the measurement directly by performing the Fourier Transform on the data directly and then integrating the F.T. results.

The machine, after establishing a particle velocity/mobility distribution at high frequency, then measures a low frequency and creates a raw low frequency particle velocity/mobility distribution.

The machine then calculates a modified/corrected/particle velocity/mobility distribution in accordance with equation (d) of figure 10. This is then either displayed on a screen, printed out, data from it displayed or printed out, or the spectrum or data exported from the machine to some other device.

Figures 12A and 12B illustrate typical high and low frequency spectra measured at 100Hz and 1Hz respectively. It will be noted that the peak of

Figure 12B is sharper than the peak of Figure 12A, but shifted. A low resolution method is used to evaluate the average/mean position (centre of the peak) and overlay high resolution data about that average/central peak position).

5

It will be appreciated that the low frequency of field reversal could be using a DC field.

THIS PAGE BLANK (USPTO)

FIG 1

(PRIOR ART)

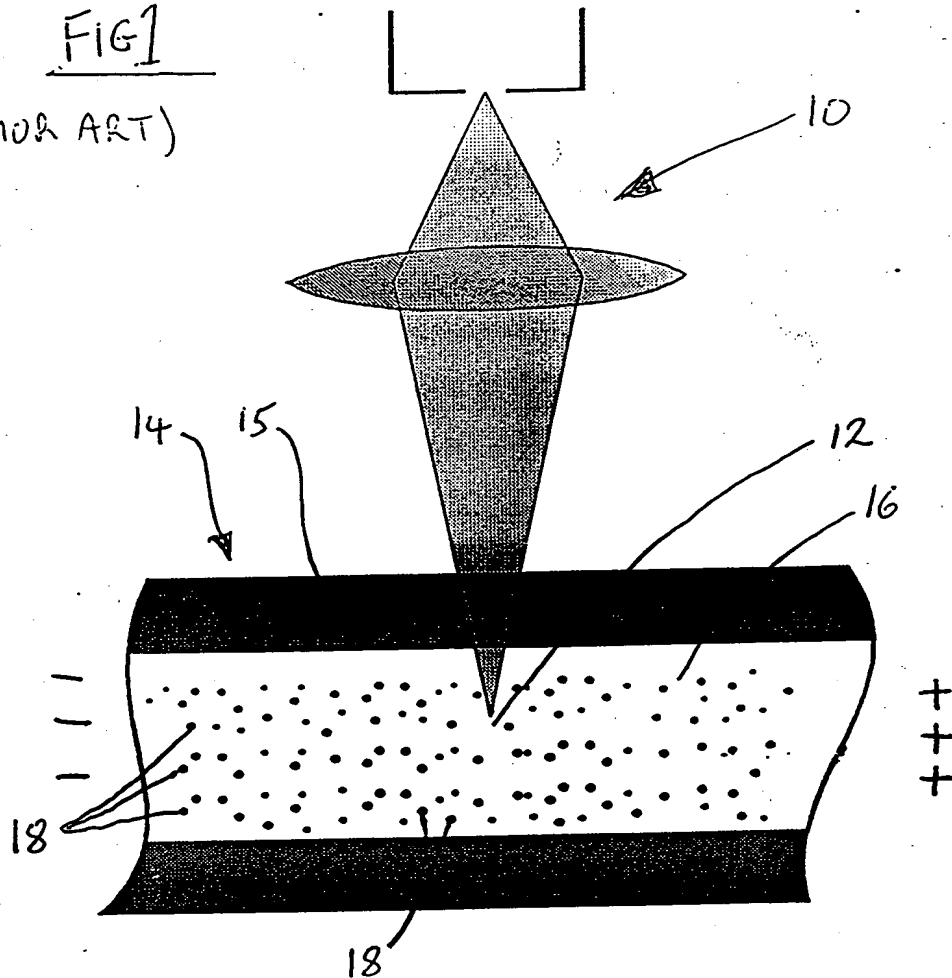
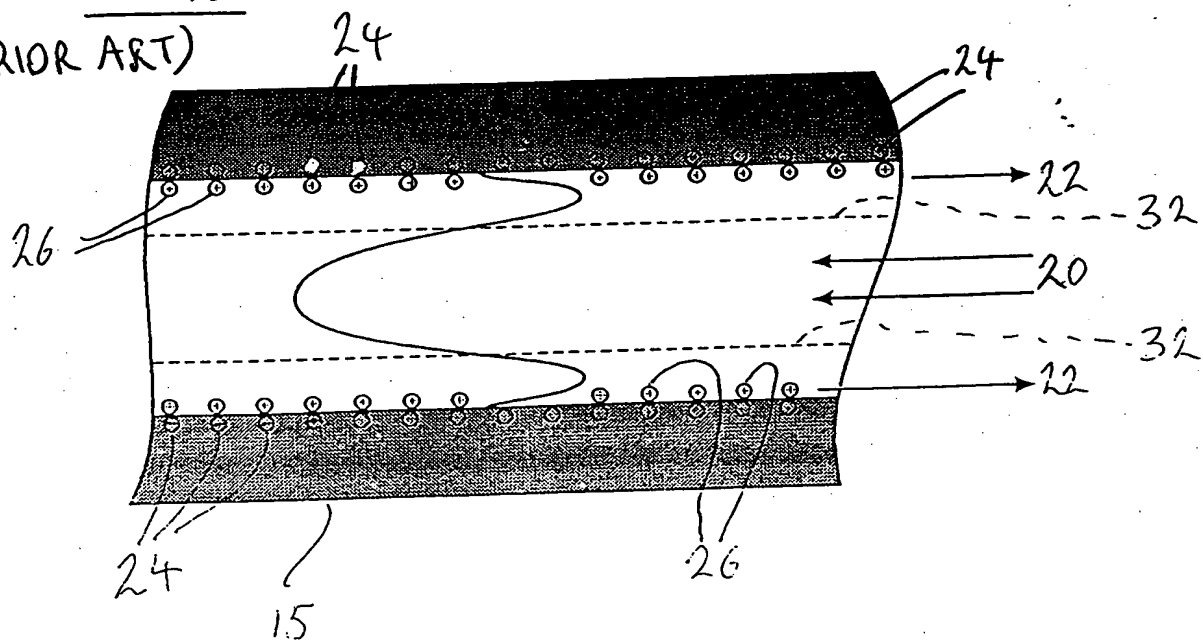
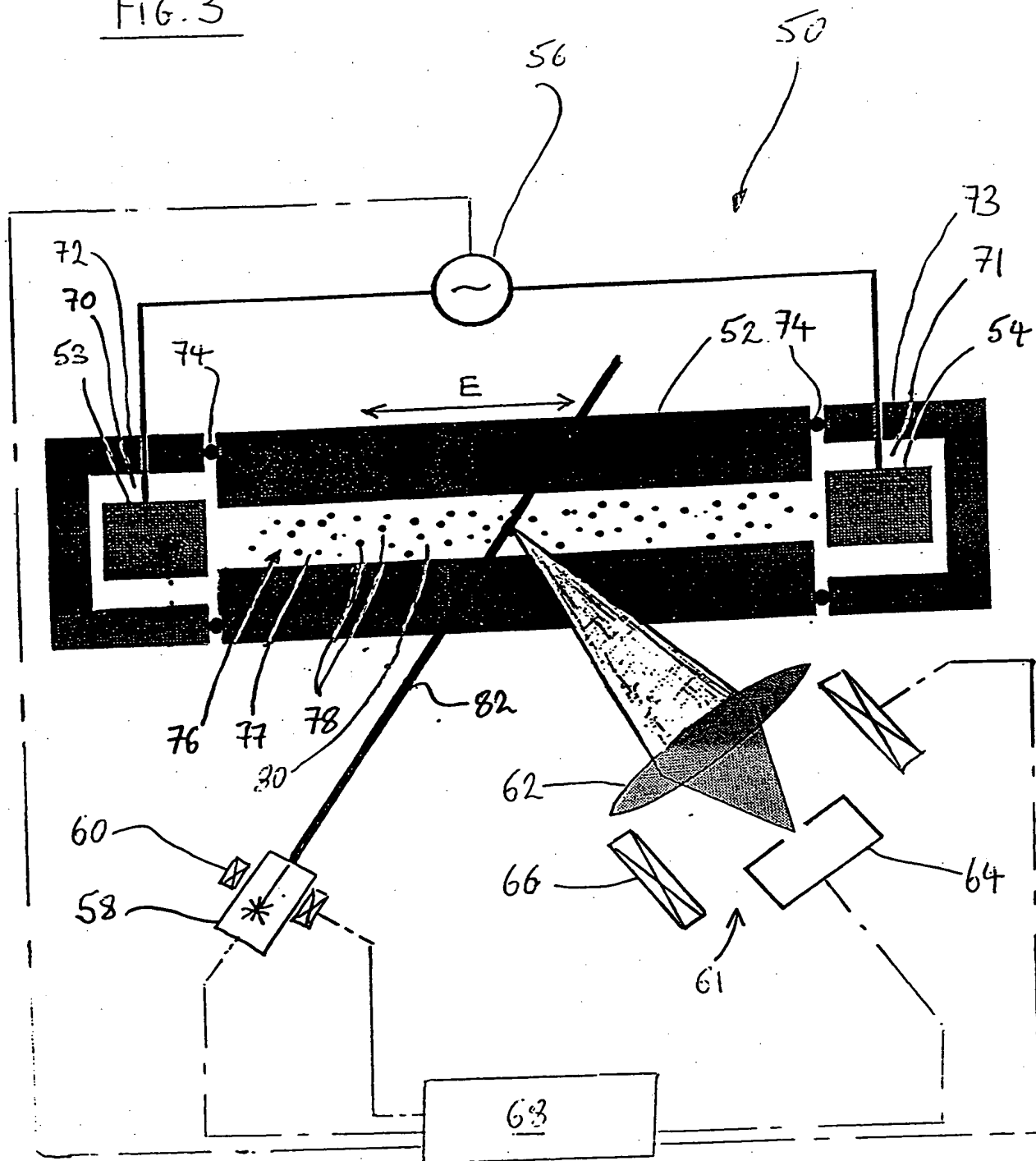


FIG.2

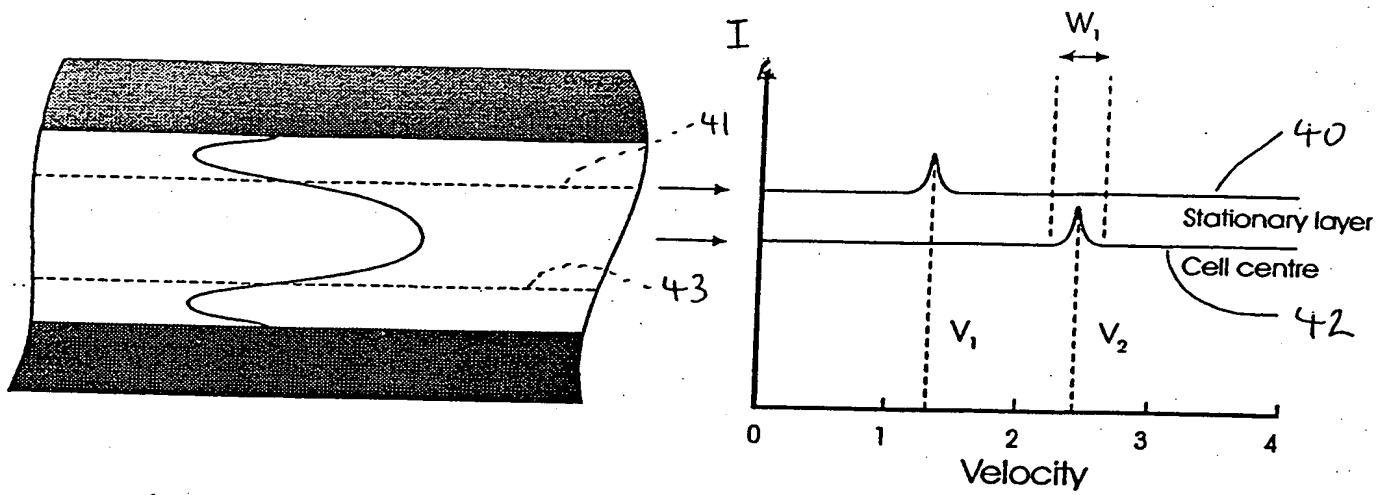
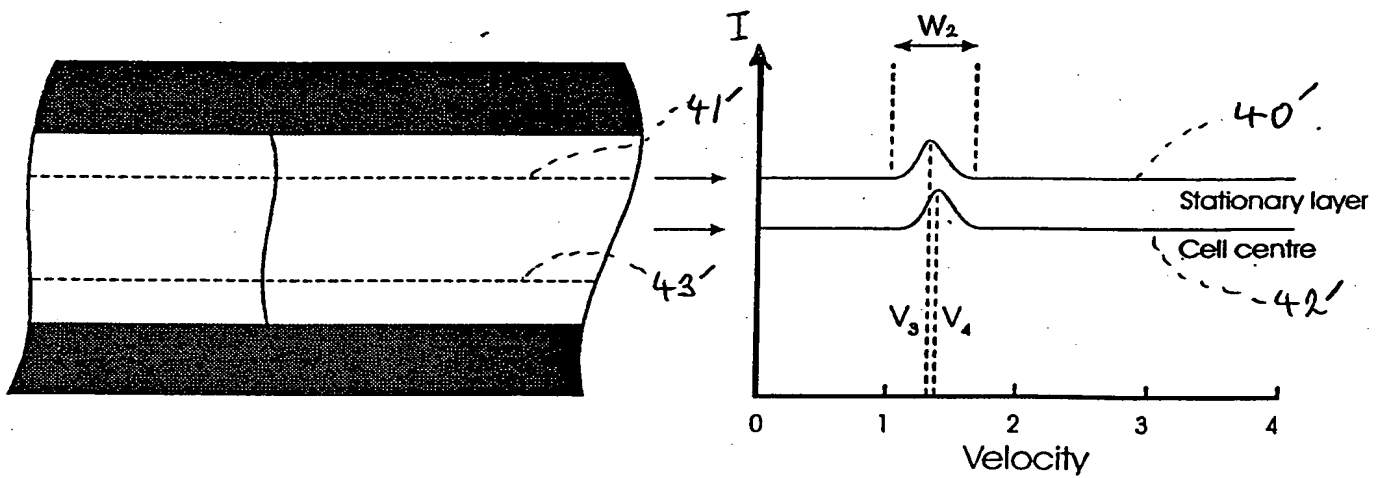
(PRIOR ART)



THIS PAGE BLANK (CSPT0)

FIG. 3

THIS PAGE BLANK (USPTO)

FIG. 4FIG. 5

THIS PAGE BLANK (USPTO)

4/9

FIG. 6

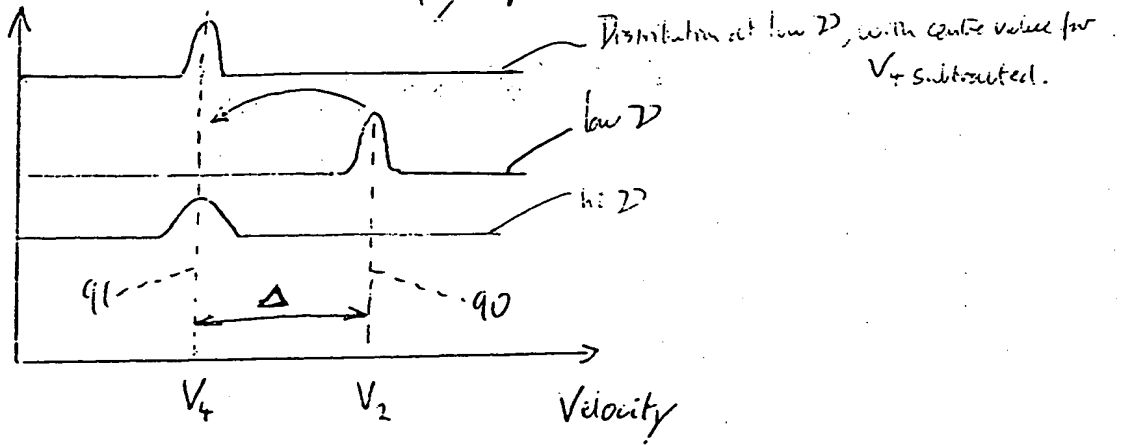


FIG. 7

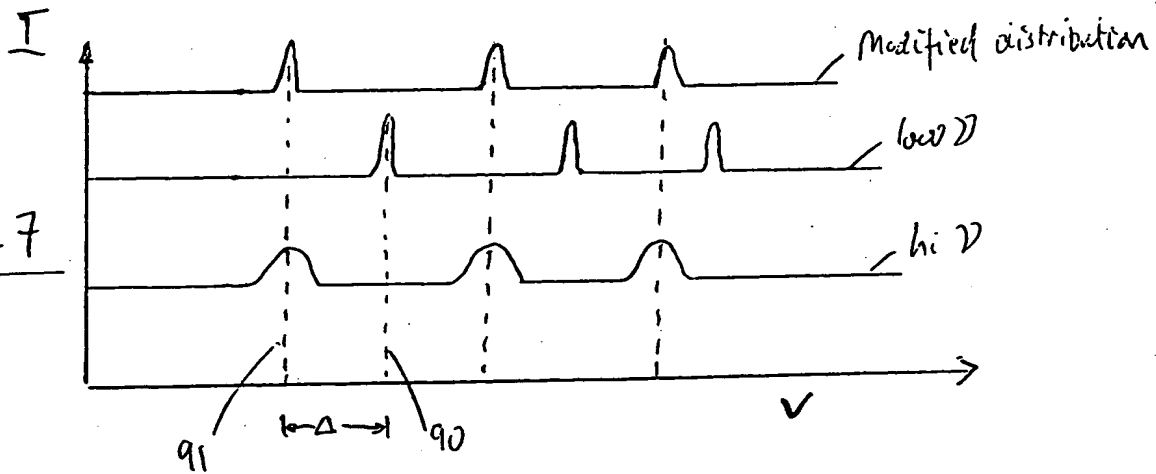
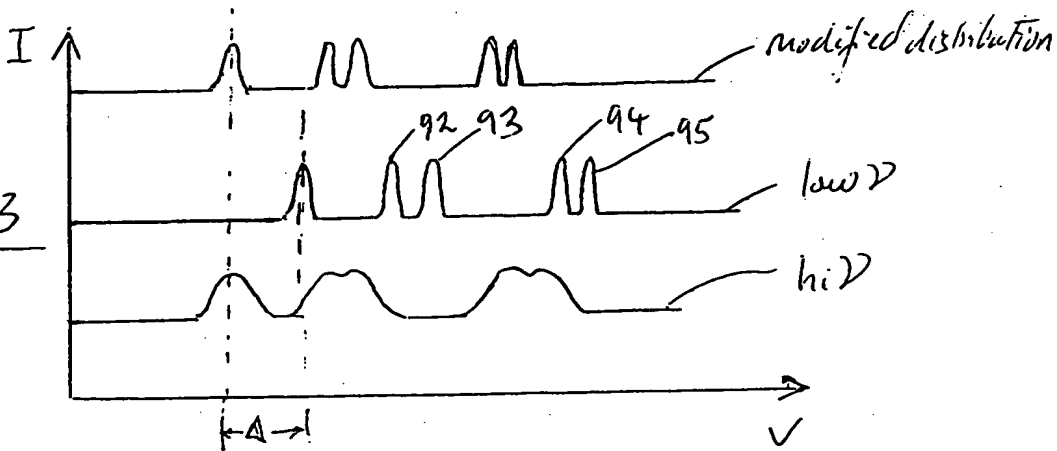


FIG. 8



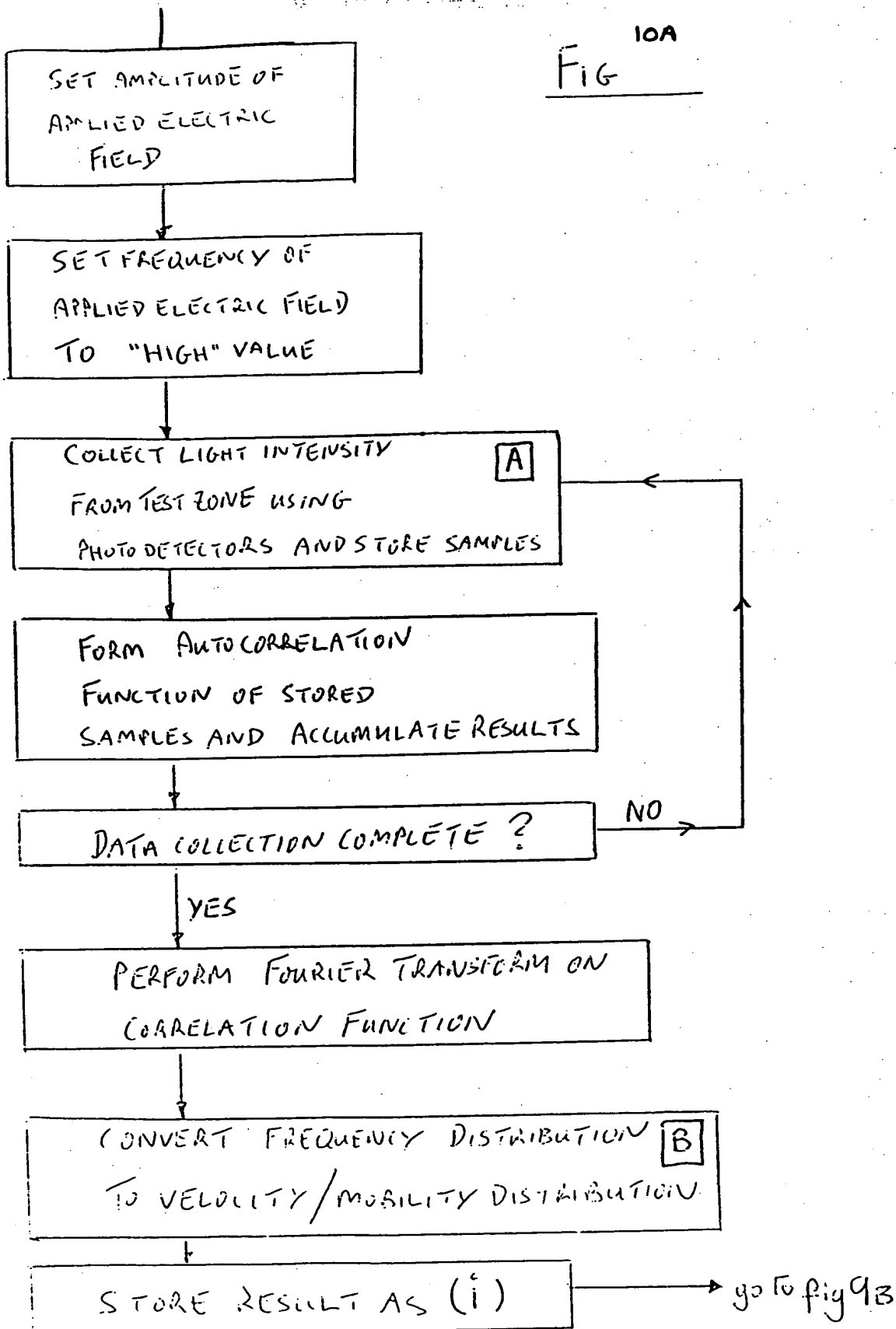
THIS PAGE BLANK (USPTO)

5/9

BEST AVAILABLE COPY

10A

Fig

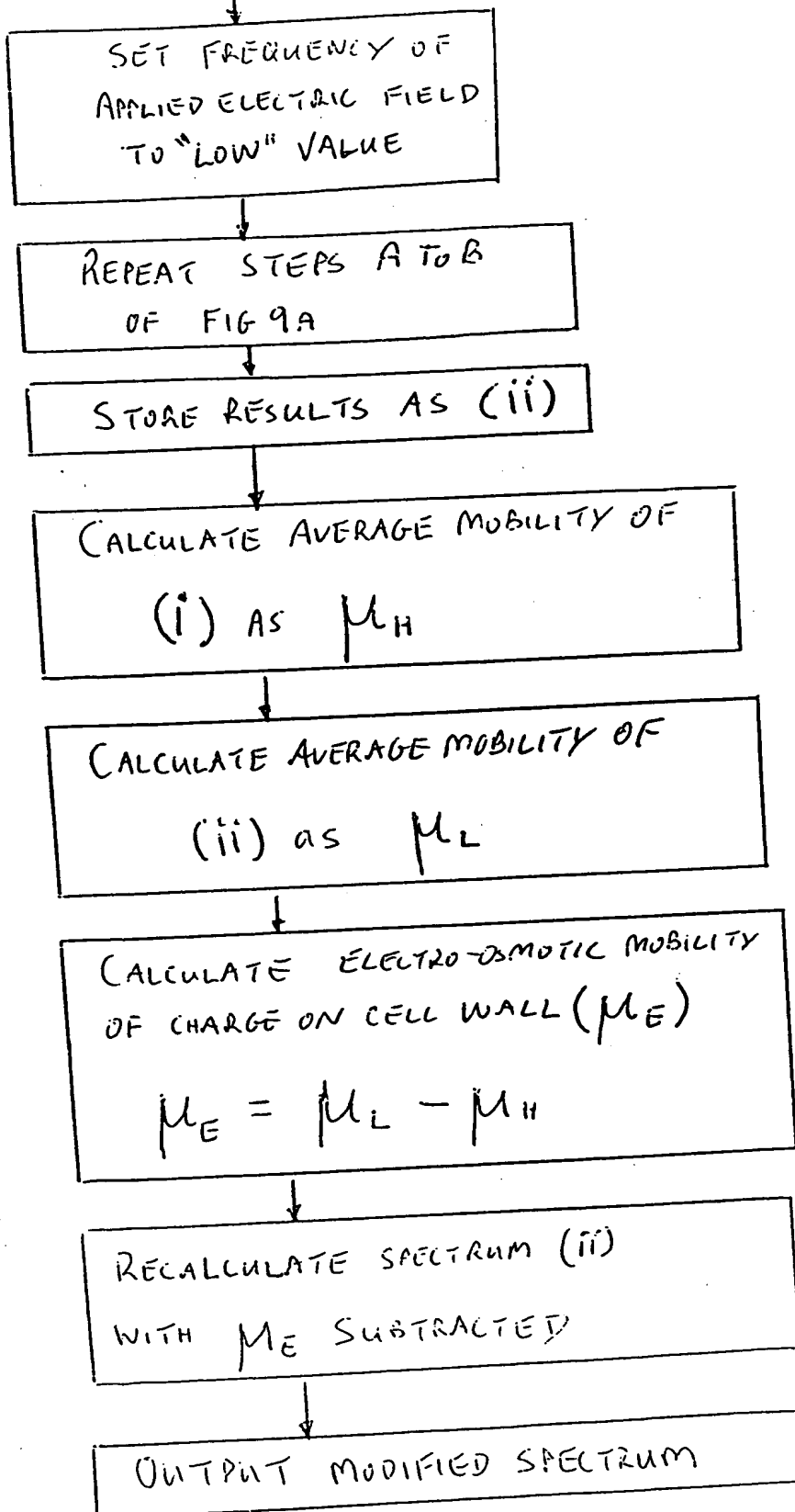


THIS PAGE BLANK (USPTO)

6/9

FIG 10B

FROM FIG 9A



THIS PAGE BLANK (USPTO)

7/9

Fig "

EQUATIONS

(a) Mobility $\mu = \frac{\text{Velocity of Particle (V)}}{\text{Electric field (E)}}$

(b)
$$\bar{\mu} = \frac{\int_{-\infty}^{+\infty} \mu f(\mu) d\mu}{\int_{-\infty}^{+\infty} f(\mu) d\mu}$$

where $f(\mu)$ = mobility spectra

(c) electroosmotic mobility $\mu_E = \bar{\mu}_L - \bar{\mu}_H$

where μ_L = particle mobility at low frequency applied electric field.

and μ_H = particle mobility at high frequency applied electric field.

(d) $f'(\mu) = f_H(\mu + \mu_E)$

where $f'(\mu)$ is the corrected form of the particle mobility distribution function.

THIS PAGE BLANK (USPTO)

8/9

FIG. 12A

BEST AVAILABLE COPY

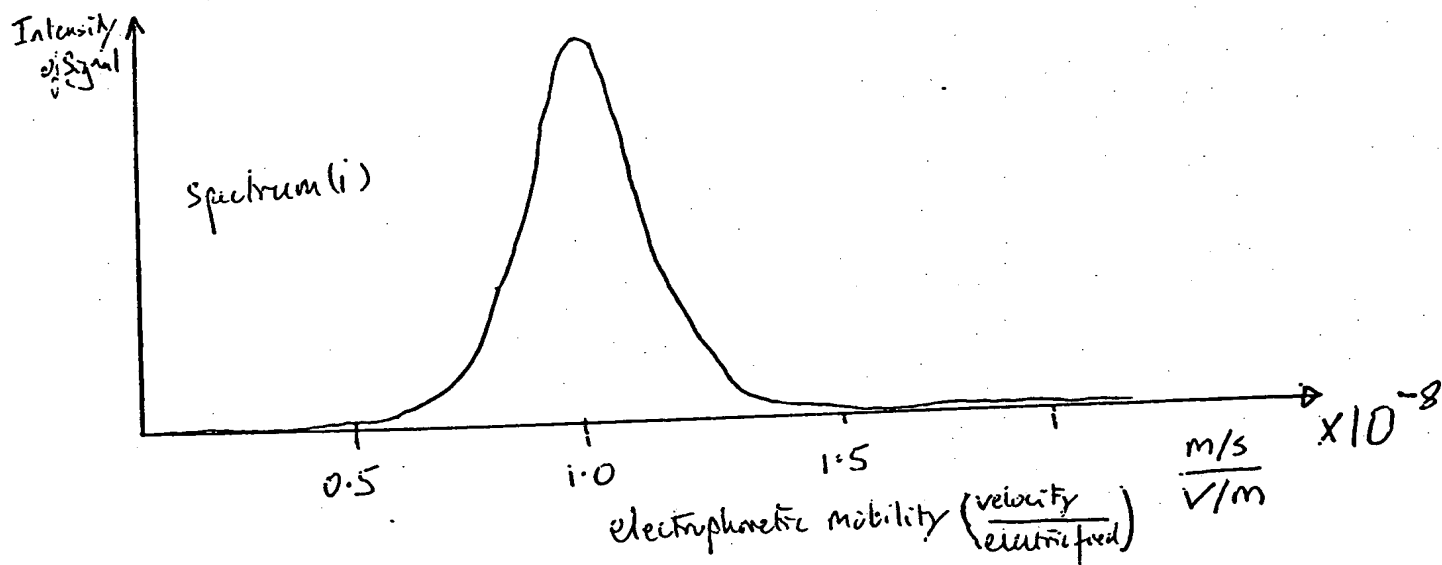
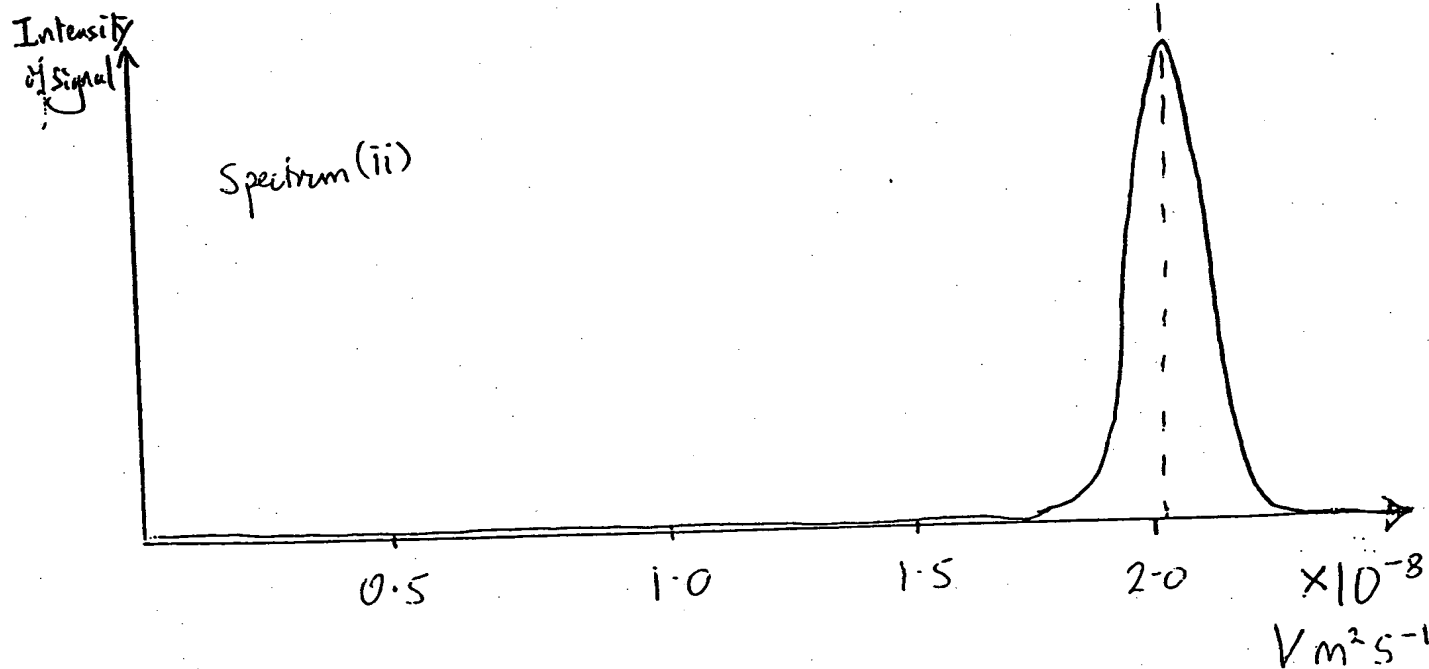


FIG 12B



THIS PAGE BLANK (USPTO)

9/9

BEST AVAILABLE COPY

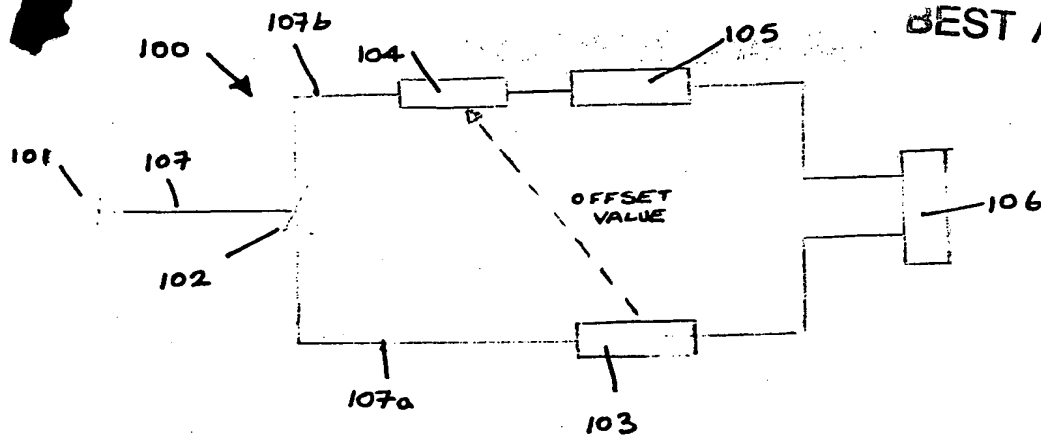


Figure 9

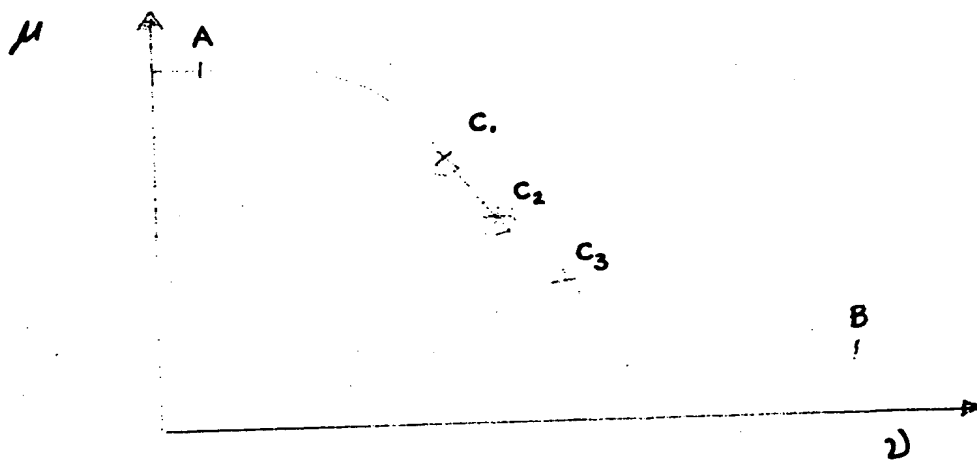


Figure 13

THIS PAGE BLANK (USPTO)